Docket No.: 2016-4000 US3

Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : STEINMAN, ET AL.

Serial No. : 08/261,537 Group Art Unit: 1808

Filed : June 17, 1994 Examiner: B. Lankford

For : METHOD OF IN VITRO PROLIFERATION OF DENDRITIC CELL PRECURSORS AND THEIR USE TO PRODUCE IMMUNOGENS

Assistant Commissioner of Patents
and Trademarks
Washington, D.C. 20231

DECLARATION UNDER 37 CFR § 1.131

I, Ralph M. Steinman, declare that:

1. I am presently Professor and Senior Physician of the Laboratory of Cellular Physiology and Immunology at Rockefellar University, 1230 York Avenue, New York, New York 10021-6319.

2. I am one of the inventors of the above-identified patent application, along with Gerold Schuler and Kayo Inaba.

3. I have read and understand the Office Action dated October 19, 1996 in which the Examiner cited Sallusto and Lanzavecchia, Journal of Experimental Medicine,

179:1109-1118, April 1994 in support of a rejection under 35 U.S.C. § 103 as teaching the use of granulocyte macrophage colony structuring factor (GM-CSF) and interleukin 4 (IL-4) to culture dendritic cells.

4. The instant application discloses and claims the culturing of proliferating dendritic cells in the presence of GM-CSF and an inhibitor of maturation of non dendritic cell precursors, such as IL-4. Culturing of dendritic cell precursors in the presence of GM-CSF typically results in proliferating dendritic cells clustered in an adherent "cell ball" (see the instant specification: Examples 1-8, pages 50 to 101). The addition of an inhibitor of maturation of non dendritic cell precursors facilitates the growth of the proliferating dendritic cell balls.

5. As a co-inventor in the above identified application, I communicated with Drs. Schuler and Inaba regarding several aspects of the instantly claimed invention. Such a communication is illustrated in the attached facsimile letter (attached as Exhibit 1) from Dr. Schuler to myself.

6. The photocopy of the facsimile letter (attached as Exhibit 1) sent to me by Dr. Schuler, was received by me in the United States prior to March 24, 1994. This describes the addition of IL-4 to proliferating cultures of dendritic cells in GM-CSF. Page 2, lines 10-16. The letter details how the addition of IL-4 inhibits the formation of non-dendritic cells (the so called "bad balls") but surprisingly does not inhibit the formation of

proliferating dendritic cell balls. Therefore, our invention as disclosed and claimed in the present application was complete in the U.S. prior to the publication of Sallusto and Lanzavecchia. Moreover, my receipt of this facsimile letter, which describes the culturing of dendritic cells in the presence of GM-CSF and IL-4, confirms my involvement in the United States in the making of this invention.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Aug 2, 1996

RALPH M. STEINMAN, M.D.

Telefax page 1 of 2

To
Ralph M. STEINMAN, M.D.
The Rockefeller University
Laboratory of Cellular Physiology and Immunology
1230 York Avenue
New York, NY 10021, USA
FAX: ..212 - 327 - 8875

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Dear Ralph,

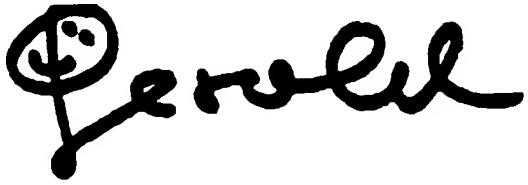
I would like to give you a brief update on a few things .

As you know we have been successful to culture DC as proliferating balls from cord blood as well as blood and bone marrow from patients that had been treated with G-CSF or GM-CSF. For that we had cultured the MHC class II and CD3 negative fractions of PBMC obtained by panning in GM-CSF +/- several other cytokines, and got "our" proliferating DC balls as observed in the mouse. When we cultured such fractions which we had obtained from PBMC of normal, healthy individuals we got - as I had already told you - proliferating balls within a few days. These balls looked like developing DC balls in overall appearance including some initial veils at the edges, yet the balls did not develop further and even started to die. When we learned from Una that freshly isolated DC need monocyte conditioned medium for staying viable we wondered whether we had depleted monocytes via their Fc receptor as a side effect of our panning procedure. One should not forget that for the latter we use whole anti-murine Ig antibody for coating petridishes and pan the cells at room temperature at least for a while. Although we planned and still plan to test panning using a $F(ab')_2$ anti-murine Ig for panning (as well as using magnetic beads as an alternative) I thought it might be useful while waiting for the ordered antibody to try the simple protocol I had used for mouse blood. So we plated exactly as described in the mouse PBMC at 1×10^6 / ml / 24 well + GM-CSF or GM-CSF plus TNF alpha, and transferred the NAF after 1 day. As in the mouse (one

should note that I myself always got the balls mainly in the adherent fraction in contrast to Kayo who presumably washed more vigorously) we got nice proliferating DC balls although already on day 3 ! Then we repeatedly observed - with GM-CSF more rapidly than with GM-CSF plus TNF alpha - that the balls switched and turned into what we have been calling "bad balls" in our numerous experiments with cord blood and GM-CSF / G-CSF blood. Would´nt there be my experience from all these experiments and would´nt I have looked at the cultures twice a day on 7 days a week we for sure had missed that the switch occurs virtually overnight. Basically we had observed this switching phenomenon though not so impressively in our experiments with blood from G-CSF or GM-CSF treated patients and rarely even with cord blood. Taking off the non-adherent fractions retards the phenomenon also with normal blood. As IL-4 has been described to inhibit macrophage colony formation [Jansen,J.H. et al. Inhibition of human macrophage colony formation by interleukin 4, J.Exp.Med. 170:577-582 (1989)] we tried to add IL-4 to prevent macrophage development or switching to "bad balls". Remarkably this was indeed the case, and even more surprisingly the DC balls were not affected or inhibited. So IL-4 is clearly not essential for DC development yet is useful via blocking macrophage development. We are now trying to perform basic functional tests and phenotyping

Given these recent developments I would be grateful when you could let me know when I could reach you by phone as I would like to discuss how we should now proceed in the human DC project. As planned for 2 months (at least) I will try to write the DC culture stuff together.

Sincerely Yours,



Gerold SCHULER, M.D.
Dept. of Dermatology
University Hospital
Anichstr. 35, A-6020 Innsbruck, Austria / Europe